RESEARCH

Recovery of Imidazolinone-Resistant Hard Red Wheat Lines Following Imazamox Application

Bradley D. Hanson,* Lynn Fandrich, Dale L. Shaner, Philip Westra, and Scott J. Nissen

ABSTRACT

Imidazolinone-resistant hard red wheat (*Triticum aestivum* L.) cultivars are occasionally injured by imazamox applications because a portion of the acetolactate synthase (ALS) remains susceptible to the herbicide. The growth and enzyme activity of two groups of hard red wheat near-isolines with spring or winter growth habit were examined following imazamox application. Each group of near-isolines contained a susceptible cultivar and cultivars with the imidazolinone-resistant trait on either the B or D genome. The spring wheat group also contained a line carrying both the B and D genome copies of the resistance gene. In whole plant experiments, growth of all single-gene resistant lines was delayed by both 35 and 105 g ha^{-1} imazamox while the two-gene line was delayed at only the highest rate. There was a herbicide rate effect on biomass accumulation but no differences among genome locations in the single-gene resistant lines or among spring vs. winter growth habit. On an ALS enzyme basis, however, there were differences among B- vs. D-genome resistance and between winter and spring growth habit. Spring wheat cultivars with the B-genome resistance had greater reductions in ALS activity compared to the D-genome cultivars, while in winter wheat, B- and D-genome lines responded similarly. Differences among genotypes existed in the recovery of ALS activity in imidazolinone wheat but other factors also likely influence the injury occasionally observed in the field.

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Abbreviations: ALS, acetolactate synthase; DAT, days after treatment.

Hard red wheat (*Triticum aestivum* L.) is grown on more than 12 million hectares in the Great Plains region of the United States and weed control is an important management consideration for wheat producers each year (USDA–National Agriculture Statistic Service, 2006). Weeds, particularly annual grasses, can be difficult to selectively control in wheat. Development of herbicide-resistant crops has resulted in new opportunities for selective weed control in many crops including wheat, and, in some cases, has simplified weed management. Herbicide-resistant crops allow for expanded use of certain broad spectrum herbicides with favorable environmental profiles (Duke, 2005; Devine, 2005; Tan et al., 2005).

Imidazolinone-resistant (Clearfield) winter wheat was commercially released in the Great Plains and Pacific Northwest regions of the USA in 2002 and 2003, respectively, and was grown on 242,000 ha in 2006 (S. Tan, personal communication, 2006). Imidazolinone herbicides inhibit acetolactate synthase (ALS, also called acetohydroxyacid synthase or AHAS, EC 4.1.3.18), the first enzyme unique to branched-chain amino acid biosynthesis (Shaner et al., 1984). Imazamox was registered for use on

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imidazolinone-resistant wheat because of the herbicide's broad weed spectrum, limited soil persistence, and favorable toxicological properties (BASF, 2004; Shaner et al., 1996). This technology has been widely accepted by U.S. wheat producers because of the opportunity to selectively control important annual grass weeds including jointed goatgrass (*Aegilops cylindrica* Host), feral rye (*Secale cereale* L.), Italian ryegrass (*Lolium multiflorum* Lam.), and the brome complex (*Bromus* spp.) (Geier et al., 2004; Zemetra et al., 1998).

The imidazolinone-resistant trait in wheat was isolated after seed mutagenesis and is controlled by a single gene (Newhouse et al., 1992). The original mutant (FS4) and most of the early released imidazolinone-resistant cultivars carried the resistance trait on the long arm of chromosome 6 in the D genome (renamed *AhasL-D1*) (Anderson et al., 2004; Pozniak and Hucl, 2004). Using mutagenesis and backcrossing programs, wheat lines with *AhasL-B1* and *AhasL-A1* resistance were created and multiple-genome resistant cultivars are under development (Pozniak et al., 2004a; Tan et al., 2005).

Modern bread wheat is an allohexaploid ($2n = 42$) with A, B, and D diploid genomes each contributing to the total ALS production (Pozniak et al., 2004a; Poehlman and Sleper, 1995). Previous research suggests that the D genome in wheat may provide more total ALS than the B genome and the A genome may likewise differ (Hanson et al., 2006). Regardless of the precise contributions of each genome to total ALS production, the susceptible portion of total ALS activity in single- and two-genome imidazolinone-resistant cultivars is reduced following treatment with imazamox (Hanson et al., 2006; Rainbolt et al., 2005). Although a temporary reduction in ALS activity usually does not result in reduced productivity of imidazolinone-resistant-winter wheat, injury from imazamox occasionally does occur. Imazamox injury on imidazolinone-resistant wheat generally consists of minor chlorosis, dark green color of developed leaves, and delayed or stunted growth (Pozniak et al., 2004b). Imidazolinone-resistant-spring wheat cultivars appear to be slightly more susceptible to injury compared to winter wheat and do not have adequate tolerance to U.S. labeled rates of imazamox (Pozniak et al., 2004b; Tan et al., 2005; Hanson et al., 2006). Wheat injury can be influenced by high imazamox rates, surfactants, application timing, and environmental conditions (Frihauf et al., 2005; Geier et al., 2004; Stougaard et al., 2004; Hanson et al., 2005b).

When a susceptible plant is treated with an ALS inhibitor, growth quickly ceases (Shaner et al., 1984; Shaner and Mallipudi, 1991). However, the relationship of ALS activity and biomass accumulation is not well understood for plants containing both resistant and susceptible forms of the enzyme (either as heterozygous diploids or as polyploids such as wheat) (Hanson et al., 2005a; Rainbolt et al., 2005). One possibility is that after an imazamox application, activity of susceptible ALS is completely stopped, while the resistant ALS remains active. Depending on the requirements for branched chain amino acids and the amount of resistant ALS in the plant, biomass accumulation may slow or stop if these products are in limited supply. As the herbicide is metabolized over time, the activity of the sensitive form of the enzyme would be expected to begin to recover and eventually return to pre-application levels. Thus, the plant growth should likewise begin to recover as full ALS activity returns. Wheat cultivars with multiple-genome resistance or resistance on a more active genome may therefore be suppressed to a lesser degree or for a shorter duration.

One factor contributing to whole plant growth recovery could be the rate at which the sensitive ALS enzyme returns to normal activity. However, very little information exists on the relative recovery of growth and ALS activity of imidazolinone-resistant wheat treated with imazamox. Therefore, the objectives of this research were to monitor the recovery of several near-isolines of hard red imidazolinone-resistant wheat with different resistance gene locations and growth habit at a whole plant and ALS-enzyme level after treatment with imazamox.

MATERIALS AND METHODS

Seed for selected hard red winter wheat lines were obtained from public and private breeders. Wheat lines used in these experiments included both commercially available cultivars and advanced breeding selections. The specific wheat lines used included two sets of near isolines based on the hard red spring cultivar Gunner and on the hard red winter cultivar Wahoo (Baenziger et al., 2002). Each set included a susceptible parent, a B-genome resistant line, and a D-genome resistant line, and the Gunner group also contained an imidazolinone-resistant line with both B- and D-genome resistance. Each imidazolinone-resistant wheat line carried the same mutation resulting in a serine to asparagine substitution at amino acid residue 653 relative to the *Arabidopsis thaliana* consensus sequence (M. Dahmer, personal communication, 2005).

Whole Plant Growth Recovery Experiments

Four wheat seeds were planted 1 cm deep in commercial potting media (Metro-Mix 200 potting mix, Sun Gro Horticulture, Inc., Bellevue, WA) in 12 by 12 by 8 cm pots (801 True series inserts, ITML Horticultural Products, Inc., Brantford, ON). Eight pots of an individual wheat line were placed in a 25 by 50 cm flat. Plants were grown in a greenhouse under natural light conditions supplemented with 400-W sodium halide lamps to provide a 14-h daylength. Day and night temperatures were 24 ± 5 and 18 ± 3°C, respectively, and plants were irrigated with an automatic sprinkler system. At the 2.5- to 3-leaf stage, each flat containing eight pots was randomly assigned to an imazamox treatment (35 or 105 g ha^{-1} imazamox) or left untreated. Recommended field rates of imazamox range from 35 to 53 g ha^{-1} in winter wheat grown in the United States

(BASF, 2004). Imazamox treatments were applied with a cabinet sprayer calibrated to deliver 145 L ha^{-1} and included 0.25% v/v nonionic surfactant and 2.5% v/v urea ammonium nitrate. On the day of herbicide application, wheat plants in one pot from each flat were clipped at the soil surface, fresh weight was measured, and plants were dried in a 60°C oven for 24 h for zero days after treatment (DAT) for dry weight determination. Treated and untreated plants were maintained under previously described greenhouse conditions. Twice per week (3- or 4-d intervals) a randomly selected pot was removed from each flat and the plants were harvested, dried, and weighed as previously described. A total of seven biomass harvests were performed over the experimental time course (0, 3, 7, 10, 14, 17, and 21 DAT).

Each group of wheat lines (i.e., those with a common parent), along with ‘Above’ (Haley et al., 2003) as a standard, was examined in a separate experiment. All experiments were arranged in a randomized complete block design with three replications and each experiment was repeated. Treatments were in a full factorial (wheat line × imazamox rate) arrangement and each flat was consn3-0.2(37.6(m4s)-0.1(w711(111 300.2(l)-8.6(n

and (ii) 5% sulfuric acid diluted with distilled water (inhibits all enzyme activity). Acetolactate production was quantified indirectly by decarboxylating acetolactate to acetoin and measuring absorbance spectrophotometrically at 535 nm according to the methods of Westerfeld (1945). Before further analysis, all OD 535 values were corrected for background absorbance by subtracting the value obtained from the ACC 299,016 inhibition from each test solution.

Enzyme Resistance

Enzyme resistance was determined from plants that did not receive a foliar imazamox treatment. Resistant enzyme activity was calculated as OD 535 absorbance in the presence of 50 μmol L^{-1} imazamox expressed as a percentage of the no-imazamox control wells. Percent activity data were analyzed with a linear regression model designed to test the effects of wheat isoline, DAT, and an interaction term using PROC GLM (SAS Institute, 2001). Specific questions addressed by the statistical analyses were (i) is there a difference in ALS activity among the wheat isolines; and (ii) is the difference in ALS activity consistent across several time intervals? Specific linear contrasts were performed using $\alpha = 0.05$.

Total Extractable Enzyme

Extractable enzyme was determined from plants that received a foliar imazamox treatment but no in vitro imazamox. The OD 535 values from these plants were expressed as a percentage of the OD 535 values from plants that received neither foliar nor in vitro imazamox. Absorbance values were normalized by the amount of crude protein in untreated and treated plant extracts measured through a standard Bradford Protein Assay. Data were analyzed using nonlinear regression techniques (Systat Software, 2004. An exponential decay model,

$$f = y_0 + a^*\exp(-b^*x) + c^*x \quad [1]$$

where y_0 = an intercept adjustment, a and b = parameters describing the exponential decay function, x = DAT in the greenhouse, and c = a parameter describing the tailing upward slope of the function, was found to adequately fit data for all wheat isolines resistant to imazamox. Data from susceptible wheat isolines were not fit to a model because all ALS enzyme activity was inhibited by an application of 105 g ha^{-1} imazamox. Specific questions addressed by the statistical analyses were (i) is there a difference in extractable ALS among the wheat isolines; and (ii) is the difference in extractable ALS consistent across several time intervals?

Recovered Susceptible Enzyme

Recovery of the susceptible enzyme was determined mathematically by subtracting resistant enzyme activity from total activity. Total ALS activity at each time point was determined from plants treated with foliar imazamox but no in vitro imazamox. Resistant enzyme activity was determined from plants treated with both foliar imazamox and 50 μmol L^{-1} in vitro imazamox. Total and resistant enzyme activity data were adjusted for total extractable enzyme at each time point (see above). Percent susceptible enzyme activity at each time point was determined by subtracting the proportion of resistant enzyme activity from 1.00. Data were analyzed using nonlinear regression techniques

(SAS Institute, 2001). Data were fit to an exponential rise model described by

$$f = a^*(1-\exp(-b^*x) \quad [2]$$

where *a* and *b* are parameters describing the asymptote and slope of the line, respectively. Nonlinear contrasts were performed using $\alpha = 0.05$.

RESULTS

Whole Plant Growth Recovery Experiments

There were no significant interactions between treatment and experiment; therefore, data from the repeated experiments were pooled for analysis. The standard imidazolinone-resistant wheat cultivar Above responded similarly among replications and experiments and the data were not included in the analysis of the near-isolines (data not shown). Linear regression using a quadratic equation provided an adequate fit to biomass accumulation over this 21 d time course experiment (Fig. 1). In general, little to no biomass was accumulated by susceptible wheat lines treated with either 35 or 105 g ha^{-1} imazamox and regression parameters were not different from zero (Table 1). Single gene resistant imidazolinone-resistant wheat lines treated with either 35 or 105 g ha^{-1} imazamox had an initial slope estimate (DAT) that was not different from zero while the untreated plants usually had a positive initial slope. The initial slope for the two-gene resistant 'AP604' was reduced only by the 105 g ha^{-1} imazamox treatment. The change in the slope of the regression curves over time (DAT*DAT) was similar among all treated and untreated resistant wheat lines except 'AP601' (spring, D genome) treated with 35 g ha^{-1} imazamox. This treatment resulted in a significantly steeper response curve than the other single-gene resistant lines. Ise04 (i)- Tcfi0.0(a)i-17aisu1.2(i)--6.4(e)-120 11 ai1.1

Table 1. Model parameter estimates, standard errors and *P* values estimated by quadratic regression for untransformed biomass (g pot^{-1}) accumulation by seven wheat cultivars in response to foliar imazamox treatment.

Wheat line†	Imazamox	Model R^2	Parameter‡	Estimate	Standard error	*P* value
	g ha^{-1}					
Gunner (S)	0	0.88	DAT	3.12	1.12	0.0081
			DAT*DAT	0.09	0.06	0.1540
	35	0.74	DAT	1.35	0.19	<0.0001
			DAT*DAT	−0.06	0.01	<0.0001
	105	0.73	DAT	1.07	0.14	<0.0001
			DAT*DAT	−0.04	0.01	<0.0001
AP602 (B)	0	0.93	DAT	1.75	0.76	0.0274
			DAT*DAT	0.15	0.04	0.0019
	35	0.93	DAT	0.85	0.69	0.2252
			DAT*DAT	0.16	0.04	0.0002
	105	0.87	DAT	−0.36	0.50	0.4693
			DAT*DAT	0.15	0.03	<0.0001
AP601 (D)	0	0.90	DAT	2.13	0.93	0.0266
			DAT*DAT	0.13	0.05	0.0214
	35	0.88	DAT	−0.06	1.06	0.9535
			DAT*DAT	0.26	0.06	<0.0001
	105	0.74	DAT	0.89	0.93	0.3479
			DAT*DAT	0.09	0.05	0.0900
AP604 (BD)	0	0.94	DAT	2.09	0.71	0.0052
			DAT*DAT	0.13	0.04	0.0022
	35	0.91	DAT	2.69	0.86	0.0032
			DAT*DAT	0.09	0.05	0.0835
	105	0.84	DAT	1.61	1.21	0.1907
			DAT*DAT	0.16	0.07	0.0278
Wahoo (S)	0	0.88	DAT	2.76	1.00	0.0089
			DAT*DAT	0.09	0.06	0.1083
	35	0.67	DAT	1.95	0.28	<0.0001
			DAT*DAT	−0.09	0.02	<0.0001
	105	0.54	DAT	1.16	0.22	<0.0001
			DAT*DAT	−0.05	0.01	0.0004
WAH-002 (B)	0	0.94	DAT	2.15	0.70	0.0040
			DAT*DAT	0.13	0.04	0.0240
	35	0.89	DAT	0.06	0.61	0.9270
			DAT*DAT	0.15	0.04	<0.0001
	105	0.75	DAT	−0.72	0.51	0.1636
			DAT*DAT	0.12	0.03	0.0002
WAH-001 (D)	0	0.92	DAT	0.88	0.71	0.2233
			DAT*DAT	0.17	0.04	<0.0001
	35	0.90	DAT	0.76	0.57	0.1883
			DAT*DAT	0.12	0.03	0.0010
	105	0.88	DAT	−0.42	0.44	0.3375
			DAT*DAT	0.13	0.03	<0.0001

†S, ALS susceptible; B, B genome; D, D genome; BD, two-genome (B and D genomes) imidazolinone-resistance, respectively. Gunner, AP602, AP601, and AP604 have a spring growth habit while Wahoo, WAH-002, and WAH-001 have a winter growth habit.

‡DAT, days after treatment.

imazamox applications (Fig. 2). In contrast, 32% of the enzyme was active in spring wheat B-genome plants. A similar trend was observed in winter wheat plants with significantly less enzyme inhibited in plants that carried the resistance-gene on the D rather than the B genome. Furthermore, winter wheat plants had consistently greater enzyme activity than spring wheat plants for both B- and D-genome resistant plants (Table 2). Approximately 62% of the enzyme was unaffected by the herbicide application in the two-gene spring wheat (BD genomes, AP604). Comparison of single-gene wheat with a two-gene winter wheat was not possible due to a lack of available seed; however, there was greater enzyme activity for all assay points in the two-gene spring wheat than in any single-gene wheat.

Total Extractable Enzyme

Resistance-gene location, growth habit, and gene copy number also affected the amount of ALS enzyme extracted from plants treated with 105 g ha^{-1} imazamox. The foliar application initially affected the resistant and susceptible forms of the enzyme in the spring wheat BD isoline; approximately 15% more enzyme was inhibited by the foliar application compared to the 50 μmol L^{-1} in vitro application (Fig. 2 and 3). The total amount of enzyme extracted from these plants increased such that by 14 DAT the amount of extractable enzyme was similar to untreated plants (86%).

The total amount of enzyme extracted from plants with single-genome resistance (B or D genome) decreased between imazamox application and three DAT and remained constant through the rest of the experiment (Fig. 3). Differences in extractable enzyme were also observed between growth habit and between gene location within the winter wheat isolines (Table 3). Resistant and susceptible forms of the enzyme in spring wheat containing a single copy of the herbicide resistance gene were affected by the foliar overspray. Twenty-six percent of the enzyme was extracted from B- and D-genome spring wheat plants 1 DAT, and this value dropped to 14% for the B-genome wheat and 16% for the D-genome wheat by the end of the experiment. The initial amounts of enzyme extracted from single-gene winter wheat isolines differed; 36 and 57% of

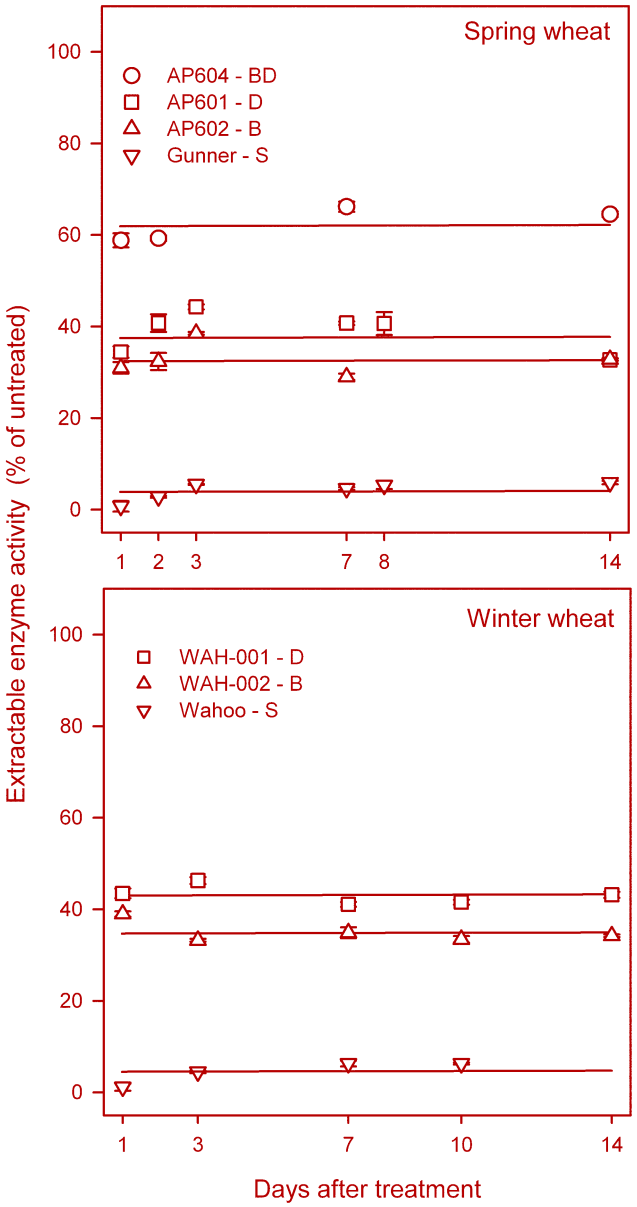

Figure 2. Imazamox-resistant acetolactate synthase (ALS) enzyme activity in hard red wheat near-isolines following an in vitro application of 50 µmol L^{-1} imazamox. Data are expressed as a percentage of the untreated controls. Lines are linear regression predictions and symbols are mean and standard error values from two independent experiments with eight replications.

Table 2. Contrasts for the regression line intercept term within and among resistance-gene genome locations in herbicide-resistant and -susceptible hard red wheat near-isolines for resistant enzyme values in response to 50 µmol L^{-1} in vitro imazamox as a percentage of untreated controls.

Contrast†	*F* value	*P* value
Within		
Winter B vs. spring B	13.03	0.0003
Winter D vs. spring D	70.76	<0.0001
Winter S vs. spring S	0.87	0.3523
Among		
Winter D vs. winter B	186.05	<0.0001
Spring D vs. spring B	56.8	< 0.0001

†Winter and spring represent wheat growth habit. B, ALS B-genome resistance; D, ALS D-genome resistance; S, ALS susceptible. Contrasts were performed using $\alpha = 0.05$.

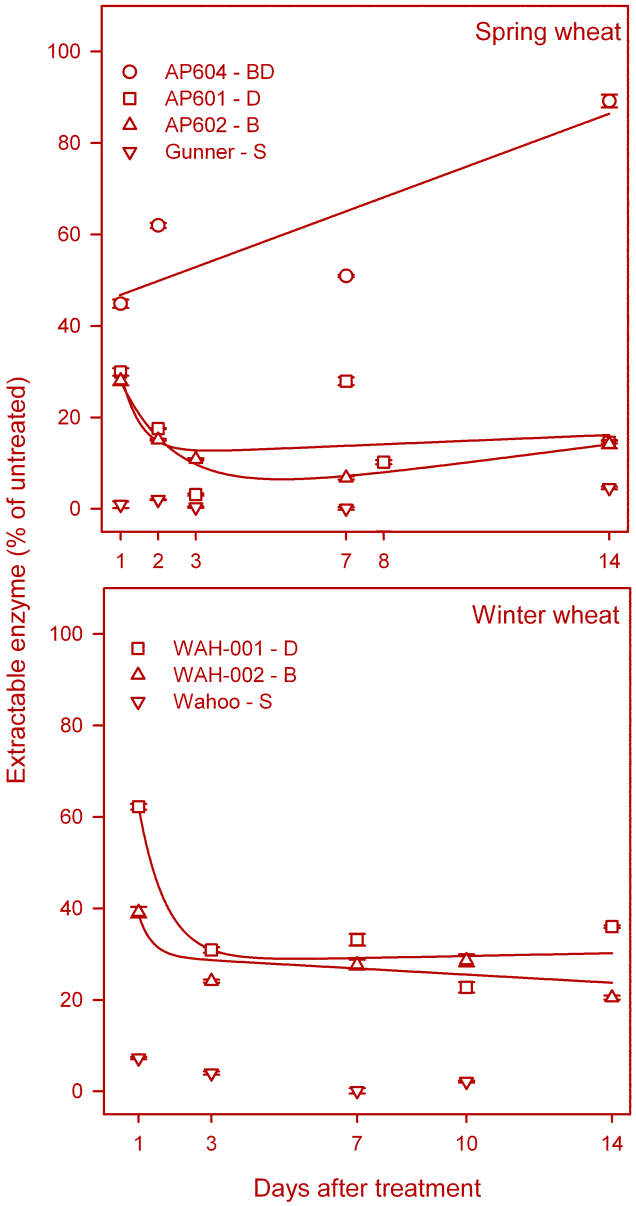

Figure 3. Extractable acetolactate synthase (ALS) enzyme activity in herbicide-resistant and -susceptible hard red wheat near-isolines following treatment with a 105 g ha^{-1} imazamox. Data are expressed as a percentage of untreated plants. Lines are predicted values based on an exponential decay nonlinear regression model (Eq. [1]) while symbols are mean and standard error values from two independent experiments with eight replications.

the enzyme was extracted from the B- and D-genome isolines, respectively, 1 DAT. The amount of enzyme extracted from these plants 3 DAT and beyond was not different within winter wheat lines, but was consistently greater than values from spring wheat B- and D-genome isolines through the same time interval.

Recovered Susceptible Enzyme

The amount of susceptible enzyme in the two-gene spring wheat (AP604) treated with 105 g ha^{-1} imazamox was constant through 14 DAT. In contrast, the amount of susceptible enzyme in single resistant-gene isoline plants (AP602,

AP601, Wah-002, Wah-001) changed rapidly over 2 d (Fig. 4), and there were no differences in responses within or among the isolines (data not shown). The amount of susceptible enzyme in B- and D-genome resistant spring wheat averaged 83% 1 DAT but there was no further change in susceptible enzyme 3 DAT. We observed also that the amount of susceptible enzyme in winter wheat D-genome plants was slower to recover between 1 and 3 DAT than the enzyme in B-genome plants (Fig. 4). Although this difference was not statistically relevant, this small biological difference, especially over a short time period may contribute to the ability of these plants to tolerate imazamox treatment. In less than 2 d, the susceptible enzyme fraction in B-genome resistant wheat reached its maximum value of 83%, but 3.5 d were required by D-genome resistant wheat to reach a similar value. This indicates that a greater portion of the total ALS activity during this critical time period is due to the resistant form of the enzyme and which likely contributes to greater tolerance to imazamox in D-genome plants.

DISCUSSION

The wheat plants used in the whole plant biomass accumulation experiments and the in vitro ALS assays were grown under slightly different conditions; therefore these data are not directly comparable. However, these two experiments provide some insights into the response of imidazolinone-resistant hard red wheat to imazamox. At the whole plant level, biomass accumulation was delayed in all single-gene resistant wheat lines by both imazamox treatments and in the two-gene resistant line only by the highest imazamox rate. During the short time course of this experiment, the initial delay in growth often resulted in reduced overall biomass production particularly in plants treated with 105 g ha^{-1} imazamox. It is difficult to predict if stunting 21 DAT observed in the greenhouse also would reduce biomass and grain yield under field conditions. In contrast to previous reports (Hanson et al., 2006; Pozniak et al., 2004a, 2004b), we did not observe marked differences between winter and spring growth habit or between B- and D-genome resistance although the two-gene wheat was injured less than either single-gene resistant spring wheat. The lack of difference between winter and spring lines in these experiments may have been due to relatively poor growth of untreated spring wheat plants caused by warmer conditions compared to previous biomass experiments

In contrast to the whole plant data, the ALS enzyme activity was strongly influenced by genome location, number of genes, and growth habit. In the absence of foliar applied imazamox, D-genome imidazolinone-resistant wheat always had higher levels of resistant ALS activity compared to B-genome cultivars and winter lines always had higher resistant ALS activity compared to spring lines. This response remained consistent across time points; however, when the plants were treated with 105 g ha^{-1} imazamox, the amount of extractable ALS activity was suppressed much more than predicted by the in vitro imazamox treatment effect, suggesting that more than just the susceptible form of the enzyme is inhibited. The amount of extractable enzyme reached its lowest point approximately 3 DAT and remained relatively constant through 14 DAT, while in the whole plant experiments, biomass accumulated at a reduced rate compared to the control plants after an initial lag period. It is not known if the changes in extractable ALS correspond directly to total ALS activity in treated plants or if the herbicide treatment reduces the extractability of otherwise active ALS enzyme. Additionally, the fraction of extractable ALS that was sensitive to imazamox rose to a plateau approximately 2 to 3 DAT indicating that the recovery of the sensitive ALS form is quite rapid, while the recovery of total extractable ALS is much slower.

The results from the ALS assay may have been complicated by the high imazamox rate used in the greenhouse. The 105 g ha^{-1} rate was chosen to ensure that whole plant symptoms were observed; however, this may have caused too much suppression

Table 3. Model parameter estimates, standard errors, and *P* values (α = 0.05) estimated by an exponential linear combination model for extractable ALS enzyme in treated plants as a percent of untreated plants.

Wheat line	Model adj. R^2	Parameter†	Estimate	Standard error	*P* value
Gunner (S)	–	–	lack of fit	–	–
AP602 (B)	0.51	y_0	–0.76	2.58	0.7682
		a	53.66	3.32	<0.0001
		b	0.66	0.10	<0.0001
		c	1.06	0.21	<0.0001
AP601 (D)	0.41	y_0	11.40	1.51	<0.0001
		a	125.36	74.54	0.0934
		b	1.92	0.64	0.0029
		c	0.34	0.14	0.0173
AP604 (BD)	0.66	y_0	–148.97	105.60	0.1595
		a	192.71	105.6	0.0691
		b	0.00	0.06	1.0000
		c	3.05	12.11	0.8015
Wahoo (S)	–	–	lack of fit	–	–
WAH-002 (B)	0.18	y_0	30.00	2.55	<0.0001
		a	128.17	1280.56	0.9203
		b	2.65	14.19	0.8518
		c	–0.45	0.24	0.0633
WAH-001 (D)	0.64	y_0	28.05	2.54	<0.0001
		a	126.23	40.47	0.0019
		b	1.31	0.38	0.0006
		c	0.15	0.25	0.5421

†The parameter y_0 is an intercept adjustment term, *a* and *b* are parameters describing the exponential decay, and *c* is a parameter describing the tailing upward slope of the function.

of ALS for us to quantify recovery in a short time frame. Field applications of imazamox often occur during periods of relatively slow growth (fall or spring) rather than during a logarithmic growth period as in these greenhouse experiments. A greater lag time between herbicide application and rapid growth periods could allow for greater imazamox metabolism and subsequent recovery of total ALS activity.

These results demonstrate that there are differences in ALS recovery among winter and spring wheat lines, and among B, D, and two-gene resistant lines. The ALS assay data did not have the same trends in recovery as the whole plant biomass recovery experiments. Although this may be partially due to environmental conditions during the biomass experiments, these data suggest that other factors besides growth habit and resistance gene location affect wheat response to imazamox. These could include the ability of the different genotypes to metabolize the herbicide to nontoxic forms, the physiological state of the plant at application, the relative expression of the imidazolinone-resistant genes, rate of enzyme turnover, and could also include other fitness traits linked to the ALS gene. Further research is needed to determine why foliar applications of imazamox reduce ALS activity to a greater extent than in vitro imazamox and how this correlates to field response of imidazolinone-resistant hard red wheat to the herbicide.

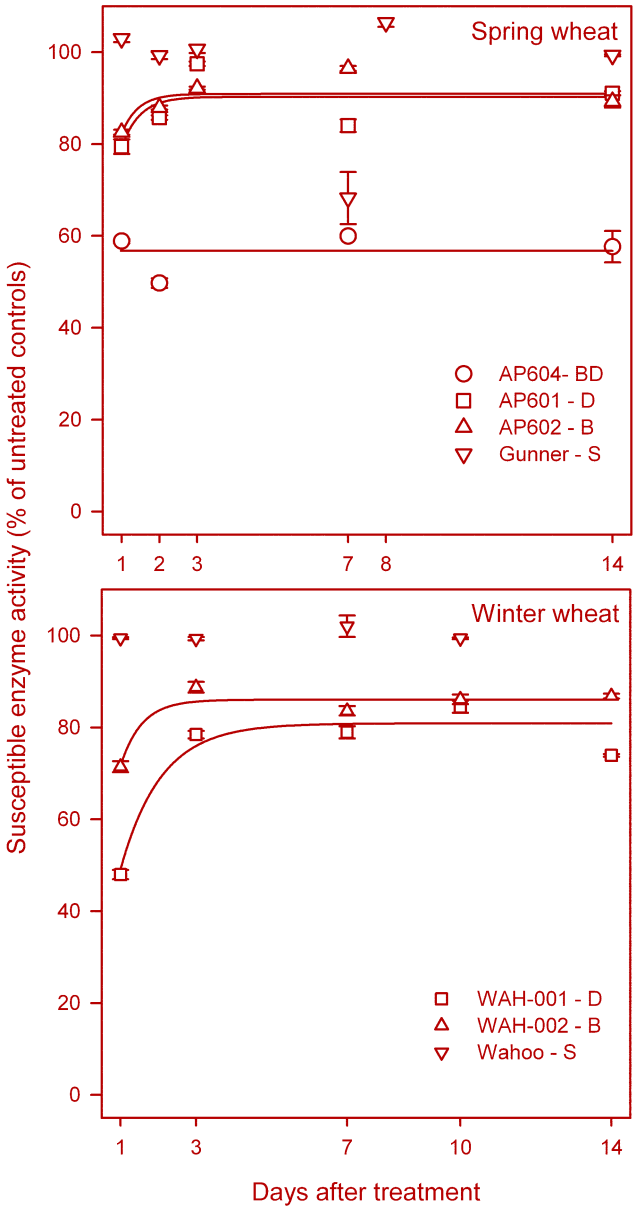

Figure 4. Recovery of imazamox-susceptible acetolactate synthase (ALS) enzyme activity in hard red wheat near-isolines following a 105 g ha^{-1} imazamox treatment. Data are expressed as a percentage of total ALS activity and are corrected for total extractable enzyme activity. Lines are predicted values based on an exponential rise nonlinear regression model (Eq. [2]) while symbols are mean and standard error values from two independent experiments with 8 replications.

References

Alvarado, S.I., A.D. Crews, P.J. Wepplo, R.F. Doehner, T.E. Brady, D.M. Gange, and D.L. Little. 1992. Discovery of a new class of herbicides: Sulfonyl carboxamides. ACS Symp. Ser. 504:75–80.

Anderson, J.A., L. Matthiesen, and J. Hegstad. 2004. Resistance to an imidazolinone herbicide is conferred by a gene on chromosome 6DL in the wheat line cv. 9804. Weed Sci. 52:83–90.

Baenziger, P.S., J.W. Schmidt, C.J. Peterson, V.A. Johnson, P.J. Matern, A.F. Dreier, D.V. McVey, and J.H. Hatchett. 2002. Registration of 'Wahoo' wheat. Crop Sci. 42:1752–1753.

BASF. 2004. Beyond product label. BASF Corp., Research Triangle Park, NC.

Devine, M.D. 2005. Why are there not more herbicide-tolerant crops? Pest Manage. Sci. 61:312–317.

Duke, S.O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Manage. Sci. 61:211–218.

Frihauf, J.C., S.D. Miller, and C.M. Alford. 2005. Imazamox rates, timings, and adjuvants affect imidazolinone-tolerant winter wheat cultivars. Weed Technol. 19:599–607.

Geier, P.W., P.W. Stahlman, A.D. White, S.D. Miller, C.M. Alford, and D.J. Lyon. 2004. Imazamox for winter annual grass control in imidazolinone-tolerant winter wheat. Weed Technol. 18:924–930.

Haley, S.D., M.D. Lazar, J.S. Quick, J.J. Johnson, G.L. Peterson, J.A. Stromberger, S.R. Clayshulte, B.L. Clifford, T.A. Pester, S.J. Nissen, P.H. Westra, F.B. Peairs, and J.B. Rudolph. 2003. Above winter wheat. Can. J. Plant Sci. 83:107–108.

Hanson, B.D., C.A. Mallory-Smith, W.J. Price, B. Shafii, D.C. Thill, and R.S. Zemetra. 2005a. Interspecific hybridization: Potential for movement of herbicide resistance from wheat to jointed goatgrass (*Aegilops cylindrica*). Weed Technol. 19:674–682.

Hanson, B.D., D.L. Shaner, P. Westra, and S.J. Nissen. 2006. Response of selected hard red wheat lines to imazamox as affected by number and location of resistance genes, parental background, and growth habit. Crop Sci. 46:1206–1211.

Hanson, B.D., D.L. Shaner, P. Westra, S.J. Nissen, and D.S. Belles. 2005b. Do environmental stresses affect Clearfield wheat response to imazamox? Proc. West. Soc. Weed Sci. 58:29.

Newhouse, K.A., W.A. Smith, M.A. Starrett, T.J. Schaefer, and B.K. Singh. 1992. Tolerance to imidazolinone herbicides in wheat. Plant Physiol. 100:882–886.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding field crops. Iowa State Univ. Press, Ames.

Pozniak, C.J., I.T. Birk, L.S. O'Donoughue, C. Menard, P.J. Hucl, and B.K. Singh. 2004a. Physiological and molecular characterization of mutation-derived imidazolinone resistance in spring wheat. Crop Sci. 44:1434–1443.

Pozniak, C.J., F.A. Holm, and P.J. Hucl. 2004b. Field performance of imazamox-resistant spring wheat. Can. J. Plant Sci. 84:1205–1211.

Pozniak, C.J., and P.J. Hucl. 2004. Genetic analysis of imidazolinone resistance in mutation-derived lines of common wheat. Crop Sci. 44:23–30.

Rainbolt, C.R., D.C. Thill, R.S. Zemetra, and D.L. Shaner. 2005. Imidazolinone-resistant wheat acetolactate synthase in vivo response to imazamox. Weed Technol. 19:539–548.

SAS Institute. 2001. SAS user's guide, version 8.2. SAS Inst., Cary, NC.

Shaner, D.L., P.C. Anderson, and M.A. Stidham. 1984. Imidazolinones: Potent inhibitors of acetohydroxyacid synthase. Plant Physiol. 76:545–546.

Shaner, D.L., N.F. Bascomb, and W. Smith. 1996. Imidazolinone-resistant crops: Selection, characterization, and management. p. 143–157. *In* S.O. Duke (ed.) Herbicide resistant crops: Agricultural, environmental, economic, regulatory, and technical aspects. CRC Press, Boca Raton, FL.

Shaner, D.L., and N.M. Mallipudi. 1991. Mechanisms of selectivity of the imidazolinones. p. 91–102. *In* D.L. Shaner and S.L. O'Conner (ed.) The imidazolinone herbicides. CRC Press, Boca Raton, FL.

Systat Software. 2004. SigmaPlot 9.0 user's guide. Systat Software, Point Richmond, CA.

Singh, B.K., M.A. Stidham, and D.L. Shaner. 1988. Assay of acetohydroxyacid synthase. Ann. Biochem. 171:173–179.

Stougaard, R.N., C.A. Mallory-Smith, and J.A. Mickelson. 2004. Downy brome (*Bromus tectorum*) response to imazamox rate and application timing in herbicide-resistant winter wheat. Weed Technol. 18:1043–1048.

Tan, S., R.R. Evans, M.L. Dahmer, B.K. Singh, and D.L. Shaner. 2005. Imidazolinone-tolerant crops: History, current status, and future. Pest Manage. Sci. 61:246–257.

USDA–National Agriculture Statistic Service. 2006. Crop production—2005 summary. Available at: www.nass.usda.gov (verified 3 Aug. 2007). USDA-NASS, Washington, DC.

Westerfeld, W.W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. 161:495–502.

Zemetra, R.S., J. Hansen, and C.A. Mallory-Smith. 1998. Potential for gene transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). Weed Sci. 46:313–317.